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EMULSIONS OF WORMSEED OIL AND OF CARBON DISULFIDE FOR DESTROYING LARVAE OF THE JAPANESE BEETLE IN THE ROOTS OF PERENNIAL PLANTS [1]

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THE PLANTS CONCERNED

Japanese iris (*Iris kaempferi*),[2] peonies (*Paeonia* spp.), and perennial phlox (*Phlox* spp.) are all extensively grown in and near the area infested by the Japanese beetle, *Popillia japonica* Newm. The acreage of these crops in nurseries growing miscellaneous perennial stock is considerable, and there are also nurseries of considerable size specializing in iris and peonies.

These three plant species are essentially different from each other in root structure. The roots of Japanese iris (fig. 1) are an impenetrable mass of coarse fibers interspersed with small quantities of soil and emanating from a hard, thick rootstock or crown. Larvæ of the Japanese beetle are found in this mass of roots and soil close up to the crown, and can be discovered and removed only by cutting, which is an obviously impractical method. The roots of perennial phlox, while coarse and heavy, are not matted to any great extent except when the soil is wet at the time of digging in November, but this condition prevails in two out of every three years in New Jersey and it is then difficult to remove any larvæ present except by washing. This operation appreciably injures the roots. In the case of the peony, the root structure is tuberous with many cavities mostly formed underground by the flower stems of the previous

[1] The writers are indebted for assistance rendered by J. W. Thomson and W. E. Fleming, investigators, New Jersey State Department of Agriculture.

[2] It is fairly probable that the Japanese beetle entered the United States in the larval form in the roots of iris from Japan.

year's growth. These cavities fill up with soil (fig. 2) and frequently afford shelter to larvæ of the beetle. The larvæ can be detected and removed only by cutting, which frequently ruins the plant.

It has been found impossible to remove all the larvæ from these plants by such ordinary expedients as shaking or washing. In 1920 and 1921 only a portion of these crops was marketed, since no method was known whereby all the larvæ present in the roots of the plants could be killed without injury to the plants themselves. Under these circumstances the writers undertook a study of this problem in an effort to discover a solution in which the plants could be dipped without injury to them for the purpose of killing any larvæ present in their roots.

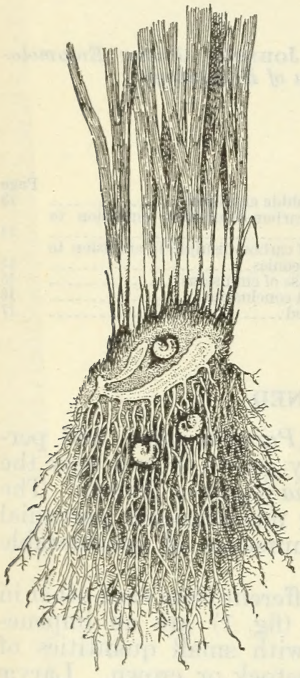

FIG. 1.—Japanese iris (*Iris kaempferi*): The matted root system with Japanese beetle larvæ interspersed

PRELIMINARY WORK

During 1920 and 1921 the writers conducted an extensive series of tests to determine the effect of various compounds upon the larvæ of the Japanese beetle and upon the roots of plants. The experimental procedure in the case of each compound was the same; larvæ were dipped for varying periods of time in filtered solutions of the compound under investigation and the mortality of the larvæ determined; potted plants, the soil of which was infested with larvæ, were *watered* with the filtered solutions and the larval mortality and the effect of the compound upon the plant were observed.

A partial list of the materials tested in this connection is given in Table 1. They include inorganic salts, alkaloids, essential oils, and representative compounds of the various organic groups. It will be observed that oil of wormseed not only controlled the larva but checked the plant only to a slight extent; carbon disulfide was somewhat more injurious to the plant. The other compounds were either innocuous to the larvæ or killed the plants. In view of these results and in consideration of the great amount of experimental work required to test out each compound thoroughly the writers decided to limit the research to wormseed oil and carbon disulfide.

TABLE 1.—*Results obtained from dipping third-instar larvæ of the Japanese beetle in various solutions*

Compound	Concentration of solution	Larvæ [1] dipped		Proportion of larvæ killed in soil [2]	Effect of solution on plants [3]
		Time in dip	Proportion killed		
		Hours	*Per cent*	*Per cent*	
Zinc chloride	5 per cent	2	0	0	Killed.
Wormseed oil	Saturated	6	100	100	Slight check.
Alpha napthol	do	1	100	75	Killed.
Benzaldehyde	do	1	66	0	Do.
Beta-napthol benzoate	do	1	0	0	Normal.
Carbon disulfide	½ saturated	1	100	100	Checked somewhat.
Carbon disulfide	Saturated	½	100	100	Checked badly.
Formaldehyde	5 per cent	1	100	0	Killed.
Furfural	3 per cent	1	0	0	Do.
Mercuric chloride	0.1 per cent	1	0	0	Injured badly.
Paraldehyde	8 per cent	1	0	0	Killed.
Pyridine	3 per cent	1	0	0	Do.
Petroleum ether	Saturated	1	0	0	Normal.
Thymol	do	1	100	90	Checked considerably.
Toluene	do	1	100	33	Checked.

[1] Larvæ not in soil.
[2] Larvæ in pots of soil (light sandy loam) watered with a volume of the solution equal to the volume of the soil.
[3] Salvia, aster, nasturtium, and chrysanthemum.

OIL OF WORMSEED (AMERICAN)

American wormseed oil (*oelum chenopodii anthelmintici*) is distilled in Carroll County, Md., from the entire cultivated plant of *Chenopodium ambrosioides anthelminticum* Linné (family Chenopodiaceae).

In the ninth edition of the U. S. Pharmacopœia the oil of chenopodium, or oil of American wormseed, is described as a volatile oil distilled from the above-named plant. The oil is colorless or pale yellow, soluble in 8 volumes of 70 per cent alcohol, and varying in specific gravity from 0.955 to 0.980 at 25° C.

In recent years producers and dealers have urged that the U. S. P. standards for this oil should be lowered, basing their argument on the fact that authentic oils obtained at the stills do not come up to the standard. However, Russell (*6*)[3] has shown conclusively that this shortcoming is due to faulty distillation, and that by distilling the herb with a large volume of steam during a relatively short period of time an oil can be produced that will meet all the U. S. P. requirements.

CHEMICAL COMPOSITION OF THE OIL

American wormseed oil (*2*) contains minute quantities of the lower fatty acids, chiefly butyric acid, and less than 0.5 per cent of methyl salicylate. Of the remainder of the oil at least 60 per cent is ascaridole, with about 5 per cent of the corresponding glycol and 30 to 40 per cent of a mixture of hydrocarbons made up approximately of cymene 15 per cent, α-terpinene 5 per cent, and a new laevorotatory terpene, 10 per cent.

Practically [4] pure ascaridole can be separated from oil of wormseed by a fairly easy process (*4*). The oil is fractionated under vacuum, the heat being kept low, for wormseed oil or, specifically,

[3] The figures (italic) in parentheses refer to "Literature cited," p. 17.
[4] From correspondence with G. A. Russell.

ascaridole, suffers a molecular rearrangement when heated to 150° C. Consequently, if a vacuum of not over 6 millimeters is employed and the heat of the bath regulated the temperature of the oil need never be brought near 150° C. and the danger of explosion, owing to sudden molecular rearrangement of ascaridole, is virtually eliminated. Practically all of the first fraction up to 80° C. will consist of terpenes; the next fraction, which is ascaridole, boils at about 95° C. at 6 millimeters pressure, and the residue in the distilling flask contains some resinified products and considerable ascaridole glycol. To obtain pure ascaridole it is sometimes, in fact almost always, necessary to refractionate the ascaridole fraction.

The principal constituent of the oil, ascaridole, $C_{10}H_{16}O_2$, has a specific gravity of 1.0024 at 25° C., a disagreeable, benumbing odor, and a disagreeable taste. Ascaridole (so called because of its action against Ascaridae) is generally conceded to be the active ingredient of the oil, although some investigators state that the terpenes and the residue containing ascaridole glycol are also active. The writers have done some work on this point, with results reported later in this bulletin (Table 6).

Inasmuch as ascaridole is essentially the active ingredient of wormseed oil from the standpoint of toxicity toward insects, it is well to purchase the oil on the basis of ascaridole content rather than on that of price. A lot of oil containing 45 per cent of ascaridole at $2.50 a pound is not as economical of the money invested as another lot of oil containing 65 per cent of ascaridole and priced at $3, since the concentration of the dip for the control of the Japanese beetle larva is based on ascaridole and not on wormseed oil.

Under these circumstances it is advisable before buying oil in quantity to determine the ascaridole content by means of the method devised by Nelson (*5*). In a cassia flask, the neck of which holds 10 cubic centimeters, graduated in tenths, agitate thoroughly 10 cubic centimeters of the wormseed oil to be tested with 60 per cent acetic acid, made by mixing 60 parts by volume of glacial acetic acid with 40 parts of water. The flask is then filled to the mark with 60 per cent acetic acid and allowed to settle. The volume of undissolved oil is deducted from 10; the remainder, multiplied by 10, gives the volume percentage of ascaridole in the sample.[5]

Wormseed oil is but very slightly soluble in water, and for that reason an aqueous solution of it has very little promise as a dip for the control of the Japanese beetle larva. Under the circumstances, probably the only practical method of regulating the concentration of oil in the dip is to make an emulsion of the wormseed oil which, when added to the water, will disperse evenly.

WORMSEED-OIL EMULSIONS

Since this emulsion must be one that will disperse in water, it follows that water must be the external phase and wormseed oil the

[5] G. A. Russell, in a letter to the writers, makes the following observations on this method of ascaridole assay: "This method is only approximate, but no other method is known. The 60 per cent acetic acid takes into solution any ascaridole glycol present in the oil, and thus the apparent percentage of ascaridole is increased. In well-prepared oils which are comparatively fresh the ascaridole glycol is present only in small amounts, so that including this in the determination of ascaridole means that only a small error is introduced, amounting to probably 4 or 5 per cent. I found that in order to get good results with this method the acetic acid solution must be made up fresh, using glacial acetic acid which has not stood in partly-filled containers for any length of time. That is the acetic acid should be fresh."

internal phase. Various hydrophile colloids such as soap, glue, gum arabic, etc., were tested in this connection as emulsifiers. In each case the colloid was dissolved in hot water, added to the wormseed oil, and shaken until the emulsion formed. In this manner 15 cubic centimeters of a 20 per cent gum arabic solution added to 10 cubic centimeters of oil gives a stable emulsion; 20 cubic centimeters of 0.5 per cent agar-agar and 5 cubic centimeters of oil produced a stable emulsion; 10 cubic centimeters of a 2 per cent glue solution added to 5 cubic centimeters of oil proved to be a very stable emulsion. Dextrin, saponin, and starch were found of little value in this connection.

While the results with miscellaneous colloidal emulsifiers as described above were satisfactory, the major part of the work was done with soap as the emulsifier, since this colloid appeared the most satisfactory of all. A commercial brand of caustic potash fish-oil soap diluted with water was mixed with the oil in varying proportions and shaken. In the majority of cases no emulsification occurred. Another procedure was then undertaken; the undiluted soap was first mixed very thoroughly with the oil, giving a so-called "miscible oil" which when mixed with water gave a perfect emulsion in the instances where sufficient soap was present. The length of time required for emulsification varied directly with the concentration of the soap.

STABILITY OF THE EMULSIONS

Emulsions were obtained with 20 cubic centimeters of oil and amounts of soap varying from 10 cubic centimeters down to 0.5 cubic centimeter. A mixture of 0.25 cubic centimeter of soap to 20 cubic centimeters of oil failed to produce an emulsion, probably owing to the fact that not sufficient soap was present to form a film at the oil-water interface.

The question therefore arose as to the optimum amount of soap. It was thought that within certain limits the greater the amount of soap present the more thorough would be the division and the smaller the size of the oil globules; in other words, the finer the water suspensions and the more stable the emulsion the greater would be the number of oil globules, the greater the surface of the oil-water interface, and, therefore, the greater the amount of soap necessary. If this hypothesis is accepted it must be assumed that there would be either an increase in the size of the oil globule with the decreased amount of soap or a diminution in the thickness of the soap film on the surface of the globule. Upon measurement it was found that there was an actual increase in the size of the globule, thus explaining the decreased stability of the emulsion with the decreased amount of soap. The measurements are given in Table 2.

TABLE 2.—*Stability of wormseed-oil emulsions prepared with potash fish-oil soap*

Emulsion number	Ingredients of emulsion			Size of globules	Remarks
	Soap	Oil	Water		
	C. c.	*C. c.*	*C. c.*	*Microns*	
1	10	20	10	1 to 12	Stable; best emulsion.
2	5	20	10	2 to 16	Stable.
3	2.5	20	10	4 to 32	Unstable.
4	1	20	10	6 to 42	Unstable.
5	0.5	20	10		Unstable.
6	0.25	20	10		Did not emulsify.

From these measurements it is apparent that there is an optimum proportion of soap above which there is on standing a marked separation of the soap from the emulsion and below which there is a tendency for the globules to increase in size to such an extent that the mixture either fails to emulsify or easily breaks after emulsification.

Soaps vary greatly in emulsifying power, some brands being useless and, in fact, two batches of the same brand may vary greatly in emulsifying power (7). Under these circumstances the writers found it necessary to test each purchase of soap before emulsifying any quantity of wormseed oil for experimental purposes. In practice, in the hands of the novice, any deficiency in the soap used might easily result in an unstable emulsion. The writers therefore made a series of tests to determine the possibility of preparing wormseed-oil emulsion by means of such standard materials as oleic acid and sodium or potassium hydroxide, according to the equation—

$$C_8H_{17}CH{:}CH(CH_2)_7COOH + NaOH = H_2O + C_8H_{17}CH{:}CH(CH_2)_7COONa.$$

In making these emulsions the oleic acid was added to the oil and shaken, then N/10 NaOH or N/10 KOH was added. The mixture emulsified immediately.[6] The various tests are compared in Table 3.[7]

In using these emulsions in practice it seems advisable either to use the oleic acid or, if a commercial brand of soap is employed, to be absolutely sure by test that the material will produce a stable emulsion.

TABLE 3.—*Preparation of wormseed-oil emulsions with oleic acid and an hydroxide*

Emulsion number	Quantities of—		N/10 NaOH	Remarks on emulsions formed
	Oil	Acid		
	C. c.	*C. c.*	*C. c.*	
1 A	20	0.12	3.76	Oil separated out in 1 hour.
2 A	20	0.24	7.56	Oil separated out in 1 hour.
3 A	20	0.40	12.60	Stable emulsion. Best of series.
4 A	20	0.80	25.20	Stable emulsion.
5 A	20	1.20	37.80	Stable emulsion.
6 A	20	1.60	50.40	Curdy emulsion. Too much emulsifier.
7 A	20	2.00	63.00	Curdy emulsion. Too much emulsifier.

Emulsion	Quantities of—		N/10 KOH	Remarks on emulsions formed
	Oil	Acid		
8 A	20	0.20	6.30	Oil separated out.
9 A	20	0.40	12.60	Stable emulsion. Best of series.
10 A	20	0.60	18.90	Stable emulsion.
11 A	20	0.80	25.20	Stable emulsion.
12 A	20	1.00	31.50	Stable emulsion.

[6] The molecular weight of oleic acid is 282.37 and its specific gravity is 0.89; it follows that 317.3 cubic centimeters of the acid will make a normal solution. Therefore 317.3 cubic centimeters of oleic acid is neutralizable by 1,000 cubic centimeters of normal sodium hydroxide, or 0.03173 cubic centimeter of oleic acid by 1 cubic centimeter N/10 sodium hydroxide. Conversely, 1 cubic centimeter of oleic acid is neutralizable by 31.5 cubic centimeters of N/10 NaOH.

[7] The writers have been guided in the preparation of these emulsions by Clayton (*1*).

TOXICITY OF WORMSEED-OIL EMULSIONS

The dip made with emulsion of oil of wormseed was tested in three different ways: (1) Larvæ, free from soil, were submerged in the dip for varying periods to determine the time necessary to kill them at various temperatures. (2) A similar series of exposures was made with larvæ embedded in soil. (3) Plants infested with the larvæ were immersed in the dip under varied conditions of temperature and length of exposure to determine the toxicity of the material to the larvæ under natural conditions and the resistance of the plants to the insecticide. The entire crop of one of the local nurseries was treated in this test, which was carried out under commercial conditions.

TOXICITY OF WORMSEED OIL TO JAPANESE BEETLE LARVÆ

The toxicity of the emulsions of wormseed oil to the larvæ of the Japanese beetle was determined by submerging the larvæ, free from soil, in the dip for varying periods of time at various temperatures. Emulsion 1 as listed in Table 2, and emulsions 3A and 9A of Table 3, were employed in these tests in the proportions of 1 cubic centimeter of ascaridole to 6 liters of water. The wormseed oil employed assayed 75 per cent ascaridole by the acetic acid method already described. The results of these tests are presented in Table 4. It will be observed that the best results were obtained when the temperature of the dip was maintained at 65° or 70° F. Lower temperatures reduced the toxicity of the material.

TABLE 4.—*Toxicity of wormseed-oil emulsions to Japanese beetle larvæ (not in soil)*[1]

Temperature of dip		Percentage of larvæ killed by immersion in dip for hours specified											
		3	4	5	6	7	8	9	12	15	18	21	24
° *F.*	° *C.*												
50	10	------	------	------	50	------	------	75	75	75	100	100	100
60	16	0	------	------	75	------	------	100	100	100	100	100	100
65	18	25	50	25	100	100	100	100	100	100	------	------	------
70	21	0	50	75	100	100	100	100	100	------	------	------	------

[1] A total of 500 larvae were used in the tests on which this table is based. In each instance here recorded the larvæ were immersed for the specific time, and the percentage of those killed is tabulated. In each case the dip contained ascaridole in the proportion of 1 cubic centimeter to 6 liters of water.

TOXIC STABILITY OF WORMSEED-OIL EMULSION

In the course of the experimental work several samples of the emulsion of various ages accumulated. These were tested under identical conditions and all on the same day in order to determine whether the stock emulsion on standing had undergone any change which might affect its toxicity. The results of these tests are given in Table 5. It will be observed that the emulsion did not decrease in toxicity within the space of 40 days. The results indicate that the toxicity will persist indefinitely if the emulsion is kept in a cool place, since the chemical change, if any, is slow.

TABLE 5.—*Comparative toxicity of stock wormseed-oil emulsions of various ages* [1]

Age of stock emulsions in days	Percentage of larvæ killed by immersion in dip for hours specified						
	5	6	7	8	9	10	15
1	50	100	100	100	100	100	100
3	75	100	100	100	100	100	100
14	100	100	100	100	100	100	100
23	100	100	100	100	100	100	100
30	75	100	100	100	100	100	100
40	75	100	100	100	100	100	100

[1] All the emulsions subjected to this test contained 1 cubic centimeter of ascaridole to 6 liters of water. The test was applied at a uniform temperature of 70° F. (21° C.). A total of about 800 larvæ were used in the tests on which this table is based.

COMPARATIVE TOXICITY OF THE INDIVIDUAL INGREDIENTS OF WORMSEED OIL

As already stated, wormseed oil when distilled under a vacuum of approximately 6 millimeters pressure can be separated by proper technique and control of temperature into three fractions consisting mainly of (1) terpenes, (2) ascaridole, and (3) a residue containing principally ascaridole glycol. However, in separating the oil into fractions for determining the toxicity of its several constituents, four fractions were made, of which the first consisted mainly of terpenes, the second of a mixture of terpenes and ascaridole, of which the terpenes constitute the major portion, the third of practically pure ascaridole, and the fourth a residue consisting principally of ascaridole glycol. Data on the relative toxicity of these ingredients are presented in Table 6.[8]

In making up the individual dips for the tests, material from each fraction was emulsified with soap, using 10 cubic centimeters of soap, 10 cubic centimeters of water, and 20 cubic centimeters of the material, as was done with the wormseed oil in making the first and most satisfactory emulsion in testing the stability of emulsions, as recorded in Table 2. For each test to 6 liters of water was added 3.67 cubic centimeters of the emulsion. It will be noticed that all the ingredients of the oil are toxic to the larvæ. The ascaridole was completely fatal to larvæ in 5 hours, the terpenes in 8 hours, and the residue in 12 hours. The results merely emphasize the fact already stated that in buying oil of wormseed it is advisable to purchase primarily on the basis of ascaridole content rather than on that of price.

[8] With respect to the ascaridole glycol of the fourth fraction, the following excerpt from a letter from G. A. Russell, by whom it was fractionated and assayed, may be of interest: "I have never done any work on the keeping qualities of wormseed oil, but Nelson examined five samples of American oil which had been shipped to Brazil and subsequently returned to the United States, all of which were at least 1 year old. He found that the distillate residues, while higher than those found in fresh oil, were not excessive, and concluded from this that the oil does not deteriorate very rapidly with age. It is my opinion that oil preserved in well-filled containers will keep without appreciable change for a period of at least 1 year. This glycol is formed by the rearrangement of the ascaridole molecules, and apparently is produced by the action of the steam on the ascaridole at the time of distillation. This may account for the high percentages of residue obtained when fractionating oils distilled by means of low-pressure steam over a relatively long period of time."

TABLE 6.—*Comparative toxicity of four fractions of wormseed oil* [1]

Fraction	Properties at 25° C.					Percentage of larvæ killed by immersion in dip for hours specified					
	Specific gravity	(A) (D)	(N) (D)	Solution in 70 per cent alcohol	Solution in 60 per cent acetic acid	5	6	7	8	12	24
					Per cent						
First	0.858	—Strong.	1.4805	None	8	50	75	25	100	100	100
Second	0.950	—8.73°	1.4760	6.5 vols	62.5	100	100	75	100	100	100
Third (ascaridole fraction)	1.0024	—2.41°	1.4720	1.4 vols	100	100	100	100	100	100	100
Fourth (residue)	1.0181	—2.53°	1.4780	0.5 vol	97	75	100	75	75	100	100

[1] Temperature of dip in each case, 70° F. (21° C.). The larvæ were immersed for the specified number of hours in the dip prepared from the fraction tested, and the percentage of those killed is tabulated. A total of about 300 larvæ were used in these tests.

APPLICATION TO LARVÆ IN SOIL AND PLANTS

The results given in Table 4 indicate the action of wormseed-oil emulsion dip upon the larvæ when the latter are removed from their habitat (the soil) and dipped. The larvæ are killed in six hours at a temperature of 70° F. When, however, the soil containing larvæ or infested plants (such as iris or phlox) is dipped in the material, it must be submerged for a longer period in order to kill all the larvæ present. The soil itself apparently absorbs the toxic material from the dip and interferes to some extent with the insecticidal action of the material upon the larvæ. This phenomenon of soil absorption and its relations to the use of soil insecticides has been discussed at considerable length in a previous paper by Leach and Thomson (*3, p. 58*), and summarized as follows:

Dipping tests indicate that certain compounds in solution, capable of producing a gas insoluble or only slightly soluble in water, are toxic to *Popillia* larvae. These compounds may be divided into two classes: (1). Compounds slightly soluble in water, e. g., carbon disulphide, thymol, mustard oil, etc. (2). compounds readily soluble in water, such as sodium sulphocarbonate and sodium ethyl xanthate. These compounds in solution, on being decomposed by organic acids, yield carbon disulphide, the active killing agent.

Saturated solutions of compounds in class 1 (about 1 to 1,000) readily kill *Popillia* larvae when the latter are removed from the soil and dipped in the solution for a definite period of time. However, when *Popillia* larvae are embedded in a soil-ball and the latter dipped in these solutions the grubs contained within the soil-ball remain unharmed. Soil adsorption, or, in other words, physical "locking up" of the compound in solution by the moisture film surrounding the minute soil particles, is the apparent reason for the failure of these relatively dilute solutions to function in soil. That portion of the compound adsorbed by the soil is apparently rendered impotent as far as its ability to produce larval mortality in the soil is concerned.

Compounds of class 2, when used in dilute solutions give results comparable to those obtained by the use of compounds in class 1. However, when compounds of class 2 are employed in relatively concentrated solutions, a quantity of the compound sufficient to produce 100 per cent mortality of *Popillia* larvae remains free in the soil after the soil particles have adsorbed the compound to the limit of their capacity.

However, in the treatment of such plants as Japanese iris, phlox, and sedum, the limitation above noted does not preclude success in killing the larvæ present in the root mass, for, while *some* soil is

present, it is confined to small amounts which can not be shaken out or otherwise removed before treatment. The presence of this small quantity of soil simply slows down the action of the wormseed-oil emulsion dip and necessitates a longer period of dipping to secure mortality of the larvæ under these conditions than is the case when the latter are entirely free from soil.

TABLE 7.—*Results obtained in dipping Popillia larvæ (in soil balls) in wormseed-oil emulsion dip* [1]

Dosage (ascaridole per 6 liters of water)	Percentage of larvæ killed by immersion in dip for hours specified				
	6	12	15	18	24
1.0 cubic centimeter	50	100	100	100	100
2.0 cubic centimeters	75	100	100	100	100

[1] The larvæ were immersed for the specified time at a temperature of 70° F. (21° C.), and the percentage of those killed is tabulated. A total of about 250 larvæ were used in the tests on which this table is based.

The results of dipping soil containing larvæ are presented in Table 7. The method adopted in this phase of the work was as follows: [9] Fifty iris plants were thoroughly shaken and the soil thus removed discarded. The plants were then cut to pieces and every vestige of soil removed and saved. This was measured by volume and averaged 7 cubic centimeters per plant. Ten cubic centimeters of soil containing a *Popillia* larva was wrapped in a small bag of muslin and the bag tied at the throat with twine. A sufficient number of such bags, each containing one larva, were used for the dipping tests the results of which are presented in Table 7. It will be noted that the 1 cubic centimeter ascaridole dosage was completely effective in 12 hours, while twice this concentration did not decrease the period of dipping necessary to secure a complete mortality. On the other hand, only six hours of dipping are required for killing the larvæ when no soil is present. This difference of six hours in the period of submergence necessary to kill the larvæ when soil is present is due to the partial soil absorption and consequent slowing up of the action of the toxic material. Were large quantities of soil present not all the larvæ could be killed even with long-sustained dipping. In practice, therefore, the large clumps of iris are broken up into several smaller ones and the greater bulk of the soil removed by thorough shaking.

During much of the fall and spring shipping seasons for iris, phlox, etc., the ground is cold. The question arose as to whether larvæ in this cold soil, when dipped, would be resistant to the insecticide. As a result of a series of experiments on this point, it was found that no difference in anything but the rapidity of killing resulted, whether the soil and larvæ were warm or cold before or after being dipped, provided the temperature of the dip itself was not lowered while the larvæ were submerged. However, the immersion of large quantities of cold soil or plants in the dip appreciably lowers its temperature and thereby reduces its toxicity. For this reason it is advisable to

[9] The infestation of iris, phlox, sedum, etc., by *Popillia japonica* in the infested area at the present time is light, not more than 5 to 10 per cent of the plants being infested. Further, these plants are expensive. These two facts render it almost impossible to obtain the preliminary data by natural means, since the procedure would involve the use and destruction (by cutting) of thousands of plants. The method here described was therefore adopted and the results checked and confirmed by the dipping of several thousand plants and their examination to determine the effect of the toxic material upon the larvæ present.

warm the plants in a room at 70° F. for 48 hours before dipping, and to keep the plants at 70° F. for 48 hours after removal from the dip, in order to promote the larval mortality.

TREATMENT OF JAPANESE IRIS

The roots of Japanese iris (*Iris kaempferi*) are mainly dug in the fall, beginning in September, and shipped immediately for planting. In September tests were made of the susceptibility of these plants to the wormseed-oil emulsion. Twelve plants were immersed for periods of 6, 12, 15, 18, and 24 hours, respectively, in a dip containing 1 cubic centimeter of ascaridole per 6 liters of water, at a temperature of 70° F. (21° C.), and the treated plants heeled in or planted in the nursery for further observation. Similar tests were made at the same temperature and for the same periods of immersion, but in a dip of twice the strength, i. e., 2 cubic centimeters of ascaridole to 6 liters of water, with the same subsequent treatment. Without exception, the plants came through the tests unhurt, and began to throw out new roots and leaf growth within a few days. The plants apparently withstand nearly twice the period of immersion in twice the concentration of dip necessary to insure mortality of the larvæ present in the roots.

TREATMENT OF PERENNIAL PHLOX

In this section plants of perennial phlox are dug in the fall, some when in full bloom to fill early orders, and the remainder from that time on until the ground freezes. Care is taken in digging to secure as much of the root system as possible, since the long roots are severed about 3 inches from the stock, cut into 1½-inch pieces, and the pieces sown in coldframes. These root cuttings begin to grow early in the following spring, and are later set out in the field to produce the year's crop. The mature plants, having been trimmed in the manner described, are packed in damp moss and placed in cold storage at 32° F. until February or early March, when they are removed, potted, and placed in the greenhouse and forced slightly for the spring trade.

It is evident that an insecticide employed to kill any larvæ present in or among the roots of this plant must be absolutely nontoxic to the roots, stock, and buds. Tests with wormseed-oil emulsion dip for the control of the larvæ in phlox roots were accordingly made at all stages of the harvesting and storage season. The results, the plants in every case being unhurt, indicate that wormseed-oil emulsion is a safe material to use as a means of killing any larvæ present in phlox during the period of harvesting and storing it.

Plants were dug when in full bloom, and separate lots, each of 12 plants, immediately dipped, all at a temperature of 70° F., but each lot for a specified time and in a dip of specified strength. Four lots were dipped for periods of 6, 7, 8, and 9 hours, respectively, in a dip containing 1 cubic centimeter of ascaridole to 6 liters of waters, and three lots for periods of 6, 7, and 8 hours, respectively, in a dip containing twice the proportion of ascaridole. All of these plants came through the treatment unhurt. Immediately after dipping they were set out in the nursery out of doors, and made a normal growth during the subsequent spring and summer, the blooms on the treated plants having in many cases a diameter of 6 to 8 inches.

Three similar lots, each of 12 plants, were dug after the first heavy frost and immersed for periods of 6, 9, and 12 hours, respectively, in a dip containing 1 cubic centimeter of ascaridole to 6 liters of water. The roots had not previously been trimmed, but, after dipping, the roots were cut off and divided into 1½-inch pieces and planted in a coldframe. They developed normally in the spring, but were somewhat slow in beginning growth. The roots of the plants of three other similar lots, each of 12 plants, were trimmed and cut into pieces, after which both plants and root cuttings were immersed in a dip like that for the other three lots and for the same periods, respectively. These root cuttings were planted in the same manner as those of the other three lots and made the same growth in the spring. The mature plants of all six lots were placed in cold storage until February 1, when they were potted and placed in the greenhouse, where their growth was equal to that of the controls and in many cases superior.

Still other plants were removed from cold storage February 1 and three lots, each of 12 plants, immersed for 6, 9, and 15 hours, respectively, in a dip containing, as before, 1 cubic centimeter of ascaridole to 6 liters of water. Twenty-four hours after removal from the dip these plants were potted and placed in the greenhouse. Their growth there was superior to that of the controls.

TREATMENT OF SEDUM SPECTABILE

The showy sedum, *Sedum spectabile*, has a coarse, matted root system and is frequently infested with the larvæ of the Japanese beetle. To test the efficacy of wormseed oil as a protective dip, four lots, each of six plants of this species, were dug in the early spring, the surplus soil adhering to the roots removed by shaking, and the four lots immersed for 12, 15, 18, and 24 hours, respectively, in a dip of wormseed-oil emulsion containing 1 cubic centimeter of ascaridole to 6 liters of water and at a temperature of 70° F. All came through the treatment without injury to the roots. At the time of dipping, the plants had made about 3 inches of top growth. This was injured by the dip and sloughed off, but on potting the plants and placing them in the greenhouse, the treated plants soon threw out new top growth and prospered, soon catching up with the controls.

VALUE OF WORMSEED OIL AS AN INSECTICIDE

The results of the experimental work which has been described in the preceding pages indicate that a dip the insecticidal basis of which is wormseed-oil emulsion is, under certain conditions, a reliable destroyer of the larvæ of the Japanese beetle, though not rapid in its action. Of all the compounds tested for this purpose it is the least toxic to plants. The cost of treatment with this insecticide is not prohibitive; 1 pound of wormseed oil, assaying 75 per cent ascaridole, will make 500 gallons of dip. It seems probable that this treatment could be utilized in many similar cases of needed control of insects.

TREATMENT OF PEONY ROOTS

Figure 2 represents a typical peony root, the root cavity being split open to show its characteristics as a hiding place for the larvæ of the Japanese beetle. In the majority of peony roots the cavities contain more or less soil, usually compact and in one solid mass; whereas in iris the soil is interspersed in very small individual amounts through

the tangled root mass, but is appreciable in the aggregate. Experimental work has shown that it is much easier to kill the larvæ in iris roots than in peony roots because of the difference in the distribution of the soil. Here, again, soil-absorption is apparently the limiting factor. A larva in the center of a cubic inch of soil is not affected to nearly the same extent by the dip as a larva in the center of a mass of soil and roots consisting of 1 cubic inch of soil mixed with 3 cubic inches of roots, when both are submerged in the same concentration of dip. In fact, the submergence of iris roots for 15 hours in a dip containing 0.5 cubic centimeter of ascaridole per 3 liters of water at 70° F. completely killed the larvæ in them; whereas twice this dosage was required for killing the larvæ in peony roots under the same conditions of time and temperature. Incidentally the peonies were not injured by a dip of this greater strength when submerged for the time stated, but the added cost of the dip, while not prohibitive, led the writers to experiment with other toxic materials in emulsion as a control for the larvæ infesting this particular plant. Of the materials tested in this connection carbon disulphide was found to be the most feasible.

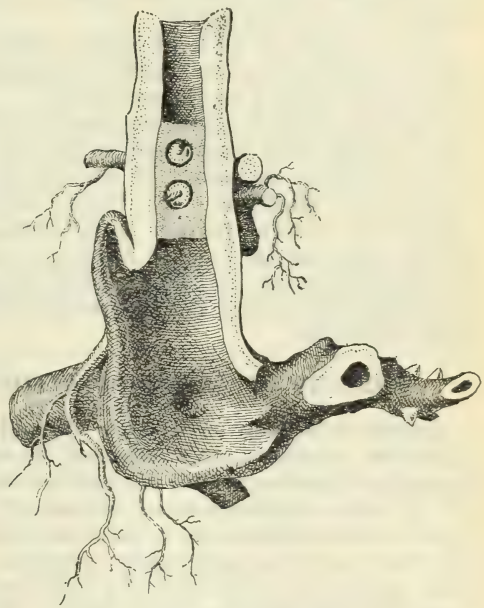

FIG. 2.—Peony root, divided longitudinally, showing infestation by larvæ of the Japanese beetle

CARBON-DISULFIDE EMULSIONS

EMULSION 1

Experimental work showed that carbon disulfide could be emulsified by soaps in general, and the writers found the old style rosin-fishoil soap to be the best for this purpose. It is a thick, heavy soap and must be heated with water to dissolve it. When it is available a stock soap solution can be made by adding 12.5 grams of rosin-fishoil soap to 87.5 cubic centimeters of water, heating until dissolved and allowing the solution to cool. Add 20 cubic centimeters of this stock solution to 50 cubic centimeters of carbon disulfide in an Erlenmeyer flask and agitate until the ingredients emulsify, which will require but a few minutes. Larger quantities, using the same proportions, may be emulsified with a butter churn or ice-cream freezer. The emulsion proper is white and has the consistency of thick cream. When added to water it disperses evenly and remains indefinitely in suspension.

EMULSION 2

Where the old style rosin-fishoil soap is not available a good emulsion may be made by mixing 0.5 cubic centimeter of oleic acid

with 20 cubic centimeters of carbon disulfide and adding N/10 KOH or N/10 NaOH until the solution is about neutral to phenolphthalein, 10 cubic centimeters of the hydroxide being ordinarily required.

Theoretically, about 18.3 cubic centimeters of N/10 potassium or sodium hydroxide is required to neutralize 0.5 cubic centimeter of oleic acid, but in tests by mixing the acid with carbon disulfide it was found that only 10 cubic centimeters of N/10 hydroxide was required. This may be due to the solvent action of the carbon disulfide upon the oleic acid, thus limiting the necessary neutralizing action of the potassium hydroxide on the oleic acid at the surface of the carbon disulphide globules, the acid in solution in the interior of each individual globule probably not being acted upon. The necessity for less than the theoretically correct amount would naturally result from this condition.

In the case of both of these carbon-disulfide emulsions water is the external phase and carbon disulfide the internal phase, with a hydrophile colloid as the emulsifier.

Carbon-disulfide emulsion was tested along three lines: (1) Larvæ (not in soil) were submerged in the dip for various periods, to determine the time necessary at various temperatures for the dip to be completely effective. (2) Peony roots infested with larvæ were dipped for various periods to determine the toxicity of the material to the larvæ under natural conditions and the resistance of the plant to the insecticide. (3) The method was tested out under commercial conditions involving the treatment of the entire crop of one of the local nurseries.

TOXICITY OF CARBON-DISULFIDE EMULSION TO LARVÆ

Larvæ free from soil were dipped in dilutions of the carbon-disulfide emulsion at different temperatures and for different periods of treatment and the results noted. Two dips were used, one of 4.2 cubic centimeters of emulsion 1, and the other of 4.57 cubic centimeters of emulsion 2, each to 6 liters of water. The results for the two were not separately recorded, the preference being slight. The results in larvæ killed for different temperatures and periods of exposure are presented in Table 8. It is evident that the optimum temperature lies between 60° and 70° F., the latter being preferable, and that much of the effectiveness of the dip depends upon a temperature not too low.

TABLE 8.—*Toxicity of carbon-disulfide emulsion to larvæ of the Japanese beetle (not in soil)*[1]

Temperature of dip (° F.)	Percentage of larvæ killed by immersion in dip for hours specified											
	3	4	5	6	7	8	9	12	15	18	21	24
50				25	25	50	75	100	100	100	100	100
60	75	100	100	100	100	100						
65	100	75	100	100	100	100						
70	50	100	100	100	100	100						

[1] The larvæ were immersed for the specified time, and the percentages of those killed are tabulated. A total of about 400 larvæ were used in these tests.

APPLICATION OF CARBON-DISULFIDE EMULSION TO LARVÆ AND PEONIES

Larvæ were placed in the cavities of the peony roots and the cavities then filled and plugged with soil. The plants thus artificially infested were dipped in various dilutions of the carbon-disulfide emulsion for various periods of time but always at a temperature of 70° F. Forty-eight hours after removal from the dip the larvæ were taken from the root cavities and the mortality determined; the plants themselves were set out in the nursery row and kept under observation for possible injury to the buds and rootstocks. This test is of interest in connection with the treatment of plants for the fall and spring shipping seasons.

Three series of treatments were tried, each series with a particular strength of solution and varying periods of time. For the first, a dip of 4.2 cubic centimeters of emulsion 1, and one of 4.5 cubic centimeters of emulsion 2, to 6 liters of water, the two considered as of equal strength, were used, and peony roots infested as just described immersed in one or the other solution for 6, 9, 12, 15, 18, and 24 hours, respectively. Plants containing in all four larvæ were submerged for each of the periods named. The peonies were uninjured by the treatment except that the bud scales were blackened by the 24-hour exposure. All the larvæ exposed for 12 to 24 hours, inclusive, were killed; for each of the other two treatments but one larva was killed, the other three coming out alive.

Dips were tried of twice the strength, 8.4 cubic centimeters of emulsion 1 and 9.14 cubic centimeters of emulsion 2, each to 6 liters of water, with immersions of 6, 12, 18, and 24 hours, respectively, four larvæ with the plants containing them being used in each case. All the larvæ were killed. The peonies were badly checked by the shortest exposure and killed by all the others.

The strength of dip was again doubled, 16.8 cubic centimeters of emulsion 1 and 18.28 cubic centimeters of emulsion 2, each to 6 liters of water being used. Four larvæ, with the plants containing them, were immersed as before for the several periods of 6, 12, 18, and 24 hours. In all cases plants and larvæ were killed.

COMMERCIAL USE OF EMULSIONS

In treating peony, iris, phlox, and sedum plants infested with Popillia larvæ the writers have found it best to pack the plants in tubs until nearly level with the top. Galvanized-iron tubs are best for this purpose since they rarely leak, as is the case with wooden tubs, and they do not absorb the toxic material from the dip.

In cold weather the plants should be allowed to warm up for 24 hours in a room kept at a temperature of 70° F. before being dipped, and the actual dipping should be performed in a room maintained at this temperature.

The water for the dip should be brought to a temperature of 75° F. In our experience, extra tubs are best for this purpose. When the water is heated to 75° F., stir in the required amount of emulsion and pour the mixture into the tubs containing the plants, being sure that *all* the plants are submerged.

The dosage and period of submergence for the various plants are as follows:

Japanese iris.—Dosage, 1 cubic centimeter ascaridole to 6 liters of water. Allow plants to remain submerged for 15 hours.

Perennial phlox.—Same dosage as for iris. Keep in the dip for from 9 to 18 hours, depending on the amount of soil present on the plants.

Sedum.—Same dosage as for iris. Dip for a period of from 15 to 18 hours.

Peony.—Dosage, 0.5 cubic centimeter carbon disulfide per liter of water. Dip for a period of 15 hours.

Care should be taken that the temperature of the dip does not fall below 65° F. at any time during the treatment. At the end of the period of submergence the plants should be removed from the dip, the latter discarded, and the plants, after draining, kept for 48 hours in a room at 70° F. Care must be taken that the plants do not dry out before or after the dipping. Plants so treated are then ready for shipment outside the quarantined area[10] *and not before.* Any chilling subsequent to the treatment should be carefully avoided, as it may lengthen the time required to kill all the larvæ.

COMMERCIAL EXPERIENCE WITH THE METHODS

During 1922 and 1923 the writers treated by the above methods approximately 10,000 Japanese iris, 10,000 perennial phlox, 1,000 sedum, and 15,000 peony, valued in all at $10,000. There have been to date no complaints from the quarantine officials or consignees.

SUMMARY AND CONCLUSIONS

Plants of the nature of Japanese iris, phlox, sedum, etc., have a matted root system, while peonies are hollow-rooted. It is impossible to eliminate larvæ of the Japanese beetle which may be present in these roots by such means as removal of the dirt, by washing or by other ordinary methods. The experimental work here outlined was therefore conducted for the purpose of evolving a chemical dip in which such plants could be immersed for definite periods of time, to make sure of killing any larvæ present, and with no resulting injury to the plant.

The results of the work indicate that oil of wormseed (American) and carbon disulfide are the best materials to use for this purpose. These substances, when added to a hydrophile colloid and water, are both capable of forming stable emulsions the toxic principle of which is retained indefinitely.

Oil of wormseed (American).—The primarily active ingredient of oil of wormseed is ascaridole, ($C_{10}H_{16}O_2$). Other ingredients of the oil are also toxic in varying degrees. For greater certainty the concentration of the dip is figured in terms of ascaridole rather than in terms of wormseed oil.

When Japanese beetle larvæ, with no soil present, are immersed for six hours in a wormseed-oil dip the concentration of which is equal to 0.5 cubic centimeter of ascaridole to 3 liters of water, the larvæ are killed, provided the temperature of the dip is maintained between 65° and 70° F. The experimental results clearly indicate that the temperature of the dip is the limiting factor in the success of this method, and under no circumstances must it be allowed to fall below 65° F. during the course of the treatment. It is advisable to maintain it at 70° F.

[10] No injury has occurred as a result of the wetting received by the plants. In two series of experiments, plants were taken out of the dip and immediately packed in damp moss. One lot was placed in cold storage for two months and the other next to a hot stove for several weeks. The first lot was normal when removed from storage, whereas the second lot made 6 inches growth in the moss.

When plants infested with larvæ are immersed in the wormseed-oil dip, it has been found that longer periods of submergence are required to insure complete larval mortality. This is due to the fact that the soil present in the roots absorbs to a certain extent the toxic material, thereby slowing up its action upon the larvæ. As a result of the research here described it is recommended that Japanese iris and sedum be immersed for 15 hours, and perennial phlox for from 9 to 18 hours, the time depending on the amount of soil present in the roots. These periods of dipping provide ample margins of safety over the time actually required to obtain mortality of the larvæ under these conditions, while the plants concerned are unaffected by the treatment.

Carbon disulfide.—In the case of peony roots it has been found advisable from the standpoint of cost to use a carbon disulfide emulsion dip. The plants should be immersed for a period of 15 hours in a dip the concentration of which is equal to 0.5 cubic centimeter of carbon disulfide (emulsified) to 1 liter of water. The same limitations of temperature apply in the use of this material as in the case of the oil of wormseed.

Commercial experience with these emulsions in 1922 and 1923, involving the treatment of 45,000 plants of this nature, valued at $10,000, indicate that when applied under Government supervision the method is satisfactory to the quarantine officials and to the nurserymen from the standpoint of cost and the safety of the plants.

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